

EXHIBIT E

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Drosophila in cancer research

an expanding role

In recent years, *Drosophila* researchers have developed powerful genetic techniques that allow for the rapid identification and characterization of genes involved in tumor formation and development. The high level of gene and pathway conservation, the similarity of cellular processes and the emerging evidence of functional conservation of tumor suppressors between *Drosophila* and mammals, argue that studies of tumorigenesis in flies can directly contribute to the understanding of human cancer. In this review, we explore the historical and current roles of *Drosophila* in cancer research, as well as speculate on the future of *Drosophila* as a model to investigate cancer-related processes that are currently not well understood.

In 1916, decades before *Drosophila* would become one of the most popular models for studying many aspects of modern biology, the discovery of melanotic tumor-like granules in mutant larvae by Bridges and Stark first suggested that flies could develop tumors¹. Later, spontaneous mutations were identified that caused animals to die at larval stages with overproliferation of certain internal tissues^{2,3}. Subsequent screens for such a phenotype were highly successful as dozens of genetic loci were recovered in *Drosophila* at a time when few human tumor suppressors had been identified^{2,4–6}. Most of the tumor-causing mutations that were identified during this time were defined as tumor suppressor genes because they behaved as recessive loss-of-function mutations⁷. Molecular characterization of some of these fly tumor suppressor genes pointed to the importance of cell–cell communication in the regulation of cell proliferation^{3,8,9} (Table 1).

Despite very promising beginnings, the fly has not received much attention as a model system for cancer research. Several factors might have contributed to this outcome. Although the

over-proliferated larval tissues and melanotic tissues that were observed in the fly mutants had some characters resembling those of human tumors, they lacked the appearance of the massive *in situ* overproliferation that is commonly associated with most mammalian tumors. Second, the molecular characterization of these early fly tumor suppressors did not demonstrate a similarity to the tumor suppressors that had been identified in humans^{10,11}. Furthermore, characterization of these fly tumor suppressor genes did not provide an obvious connection to the contemporary understanding of the processes that are involved in tumor formation, such as regulation of the cell cycle. Finally, the indiscreet classification of some *Drosophila* genes as tumor suppressors also contributed to the state of neglect by the general cancer research community. For example, inactivation of neurogenic genes causes hypertrophy of the nervous system. However, they are not tumor suppressors because the phenotype is caused by conversion of epidermal cells into neurons and not by overproliferation of neuronal tissues¹².

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TABLE 1. Cancer-related genes in *Drosophila melanogaster*

Fly genes homologous to mammalian oncogenes		Fly genes homologous to mammalian tumor suppressor genes		Fly genes that cause tumor growth or over-proliferation and their mammalian homologs	
Fly genes	Mammalian gene or product	Fly genes	Mammalian gene or product	Fly genes	Mammalian gene or product
<i>armadillo</i>	β -catenin	<i>D-APC</i>	<i>APC</i>	<i>air8</i>	S6 ribosomal protein
<i>D. Abl</i>	<i>c-abl</i>	<i>caudal</i>	<i>CDX2</i>	<i>bag-of-marbles</i>	?
<i>D. Akt</i>	<i>Akt</i>	<i>frazzled</i>	<i>DCC</i>	<i>benign gonial cell neoplasm</i>	?
<i>aurora</i>	<i>aurora 1, aurora 2, AIM-1</i>	<i>gigas</i>	<i>TSC2</i>	<i>cactus</i>	$\text{I}\kappa\text{B}$
<i>homothorax</i>	<i>Meis1</i>	<i>haywire</i>	<i>ERCC3</i>	<i>costal-2</i>	?
<i>Dcbl</i>	<i>c-cbl</i>	<i>klumpfuss</i>	<i>WT-1</i>	<i>discs large</i>	<i>hDlg, NE-Dlg</i>
<i>Dcrk</i>	<i>c-crk</i>	<i>medea</i>	<i>DPC4</i>	<i>expanded</i>	?
<i>ci</i>	<i>Gli1, Gli2, Gli3</i>	<i>mei-41</i>	<i>ATM</i>	<i>fat</i>	<i>FAT</i>
<i>cyclin D</i>	<i>cyclin D/PRAD1</i>	<i>Merlin</i>	<i>NF2</i>	<i>hyperplastic discs/l(3)xc43</i>	<i>UBE3A</i>
<i>dorsal</i>	<i>NF-κB/Rel family</i>	<i>D.NF1</i>	<i>NF1</i>	<i>lats</i>	<i>Lats1, Lats2</i>
<i>D.E2F (D.E2F+ DP)</i>	<i>E2F</i>	<i>patched</i>	<i>ptch</i>	<i>l(1)malignant</i>	?
<i>extradenticle</i>	<i>Pbx1</i>	<i>PP2A-29B</i>	<i>PPP2R1B</i>	<i>l(2)k07918</i>	?
<i>hopscotch</i>	<i>Jak kinase</i>	<i>D.PTEN</i>	<i>PTEN/MMAC</i>	<i>l(2)brain tumor</i>	?
<i>D.jun</i>	<i>c-jun</i>	<i>D.p16</i>	<i>p16(INK4a)/MTS1</i>	<i>l(2)giant disc</i>	?
<i>kayak</i>	<i>c-fos</i>	<i>Rbf</i>	<i>pRB</i>	<i>l(2)giant larvae</i>	<i>LLGL1, LLGL2</i>
<i>D.mylb</i>	<i>myb</i>	<i>spellchecker</i>	<i>hMSH2</i>	<i>l(2)talc</i>	?
<i>diminutive</i>	<i>c-Myc</i>	<i>D.Xpa</i>	<i>XPA</i>	<i>l(3)giant larvae</i>	?
<i>Notch</i>	<i>hNotch/1/TAN1</i>	<i>D.XPD</i>	<i>XPD/ERCC2</i>	<i>l(3)malignant blood neoplasm-1</i>	<i>toricrin</i>
<i>pitchoune</i>	<i>MrDb</i>			<i>l(3)malignant brain tumor</i>	?
<i>polo</i>	<i>Polo-like kinase</i>			<i>l(3)discs overgrown</i>	<i>CSNK1D</i>
<i>Polycomb</i>	<i>hPc1, hPc2</i>			<i>multi sex combs</i>	?
<i>Ras</i>	<i>Ras</i>			<i>l(1) malignant blood neoplasm</i>	?
<i>D.ret</i>	<i>ret</i>			<i>oho23B</i>	<i>RPS21</i>
<i>smoothened</i>	<i>smo</i>			<i>ovarian tumors</i>	?
<i>Src42A, Src64B</i>	<i>c-src</i>			<i>pendulin/oho31</i>	?
<i>string</i>	<i>cdc25</i>			<i>proliferation disrupter</i>	?
<i>trithorax</i>	<i>ALL-1</i>			<i>l(2) tumorous imaginal discs</i>	<i>H.Tid-1</i>
<i>D.TCF</i>	<i>TCF</i>			<i>tu(2)91k</i>	?
<i>torpedo</i>	<i>c-erbB-2</i>			<i>tumor(3)be</i>	?

A summary of functions and mutant phenotypes for the genes listed above can be found at <http://info.med.yale.edu/genetics/xu/flycancergenes>.

However, a significant number of genes that have been studied in flies have turned out to be homologs of human oncogenes and tumor suppressors¹³ (Table 1). Currently, at least 76 fly homologs of mammalian cancer genes are under intensive investigation. Studies of these *Drosophila* homologs of known mammalian cancer genes have contributed tremendously towards the understanding of the developmental functions of these genes, their actions at the molecular level and the genetic pathways in which these genes execute their functions. A list of these genes and their functions that we have compiled can be found at <http://info.med.yale.edu/genetics/xu/flycancergenes>. The knowledge that we have gained from studying these *Drosophila* genes and the biological processes in which they participate has contributed to our understanding of the mechanisms of action of their human counterparts.

Signal transduction pathways are conserved from flies to humans

Many of the extensively studied signal pathways have been shown to be conserved from flies to humans. Genetic studies in *Drosophila* have contributed significantly in revealing many of these pathways. For example, the *Ras* proto-oncogene pathway was first elucidated by studying photoreceptor cell development in the *Drosophila eye*¹⁴, as well as by studying vulval development in *Caenorhabditis elegans*¹⁵. Because of the conservation of the pathways, knowledge of fly genes and their genetic pathways are now contributing to cancer research in humans. For example, the identification of *Patched* as the tumor suppressor gene that is mutated in the nevoid basal cell carcinoma

syndrome has prompted interest in the *Drosophila patched/hedgehog* pathway for clues as to what other pathway components might act as tumor suppressors or oncogenes in humans. At least three additional members of the *patched/hedgehog* pathway have now been implicated in mammalian tumor formation¹⁶⁻¹⁹.

Although many of the signal transduction pathways that are involved in tumorigenesis are conserved from *C. elegans* to humans, the biological functions of some of these pathways might vary between different organisms. For example, in *Drosophila* and humans, the *Ras* pathway is involved both in cell proliferation and in cell fate determination, whereas in *C. elegans* it is only involved in cell fate determination^{20,21}. Moreover, cell fate determination in *C. elegans* is more lineage dependent, suggesting that some of the pathways that are involved in tissue patterning in humans will be absent or will function differently in *C. elegans*. In fact, sequencing of the *C. elegans* genome has not identified some of the key components in the *hedgehog* signaling pathway²². These findings suggest that, as a model organism, *Drosophila* has a unique role to play in the investigation of human tumor biology.

The biology of *Drosophila* provides a valid model for cancer research

The imaginal discs of *Drosophila* provide researchers with an excellent opportunity to study the development of cells whose biological properties are similar to those of mammalian cells that are susceptible to cancer. Imaginal discs are sacs of specialized epithelial cells that give rise to most of the structures in the adult fly. These discs are single-cell

layer structures that proliferate during larval stages to produce mature discs that have characteristic morphologies²³ and that differentiate into adult structures. The specialized epithelial cells that undergo proliferation and differentiation are diploid and have a cell cycle similar to that of mammalian cells, consisting of G1, S, G2 and M phases^{24,25}. The similarity between the fly and mammalian cell cycle is not restricted simply to the general organizational level; the conservation also exists at the molecular level. The fundamental cell-cycle machinery, the cyclins (A-, B-, D- and E-types) and their cyclin-dependent kinase partners (Cdk1, Cdk2, Cdk4 or Cdk6), is highly conserved between flies and mammals²⁵. The molecular conservation of the cell cycle also extends to cell-cycle regulatory components as well. For example, many mammalian cell-cycle regulators, such as the retinoblastoma protein (pRb) and E2F also have *Drosophila* homologs [i.e. RBF (Ref. 26) and dE2F (Ref. 27); Table 1]. The similarity between *Drosophila* and human cell-cycle machinery and regulatory pathways suggests that *Drosophila* can serve as a model in which to study the process of proliferation during tumorigenesis.

The mechanism of cell fate determination, which contributes to tumorigenesis in mammals, can also be studied in fly imaginal discs. The differentiation of imaginal disc cells to produce the adult structures occurs as a result of communication with surrounding cells through a combination of direct cell-cell interactions and long-range signaling^{28,29}. This mosaic type of cell fate specification is very similar to the way cell fate is determined in most mammalian tissues³⁰. It is clear that most of the molecular pathways that are involved in cell fate determination are conserved between flies and mammals. For example, the Notch transmembrane receptor has been shown to govern cell fate choice in *Drosophila* and vertebrates through a similar cell-cell communication mechanism³¹. Interestingly, mutations in human *Notch* homologs have been implicated in T-cell acute lymphoblastic leukemia/lymphomas³². Recently, it has also been demonstrated that altered *Notch* activity in *Drosophila* can result in overproliferation³³. Thus, it appears that the biochemical pathways and the processes that regulate cell fate determination are conserved from flies to humans.

In addition to the imaginal discs, the *Drosophila* embryo also provides an excellent system for the study of cell proliferation during development. Since both developmental events and cell cycle progression during *Drosophila* embryogenesis have been well documented, this system provides advantages for gaining insight of mechanisms that coordinate cell proliferation and other developmental events³⁴. The *Drosophila* ovary is also a great system to dissect the developmental regulation of cell proliferation, especially for the germline stem cells^{35,36}.

Using *Drosophila* genetics to study tumorigenesis

Recent advances in experimental techniques now offer unique advantages to examine the developmental context of cancer-causing genes. For example, the effects of ectopic gene expression can be studied easily in the fly. This is useful for studying the etiology of tumors because oncogenes are often either aberrantly activated (i.e. *Ras*) or overexpressed (i.e. *cyclin D*). Fly researchers can study the biology of gene overexpression without killing the animal by expressing the gene of interest ectopically using a specific promoter. The fly system is unique in that it offers

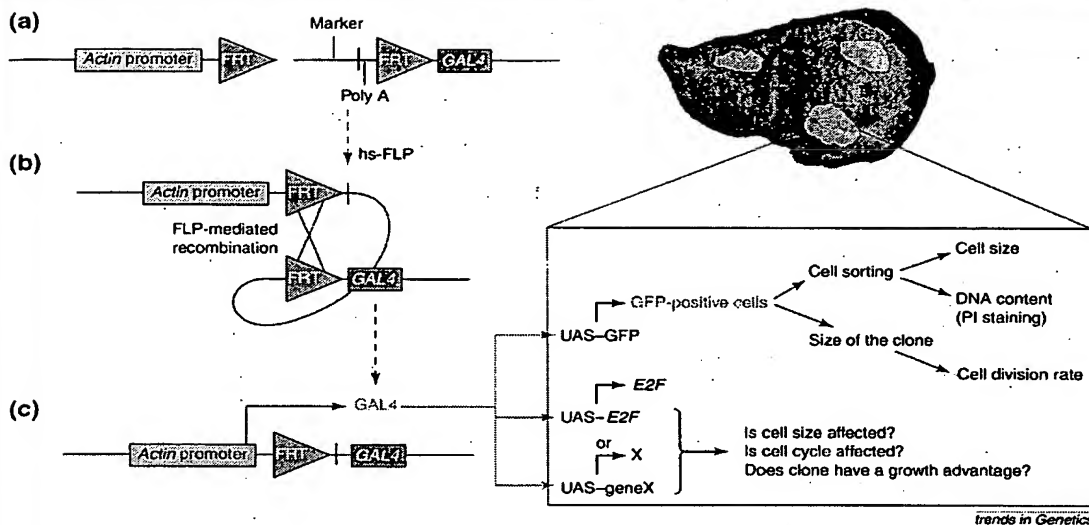
a wide range of well-characterized promoters to choose from, including ubiquitous promoters (such as the heat-shock or actin promoters) or tissue-specific promoters (such as neuronal specific or eye-specific promoters). Expression can also be controlled temporally by inducing a heat-shock promoter during a time range of interest. The diversity of available tissue-specific promoters allows the overexpression of a gene of interest to be studied without causing lethality. Similarly, a gene can be modified *in vitro* to reflect oncogenic mutations, such as deletions or point mutations, and assayed for oncogenic activity *in vivo*; this technique has been used to confirm that a mutant *Ret* gene that is found in multiple endocrine neoplasia 2B (MEN2B) tumors is hyperactivated³⁷.

Introduction of the yeast UAS/GAL4 system into the fly has made such ectopic expression studies easier and more versatile³⁸. With the UAS/GAL4 system, only the construction of a UAS-cDNA construct that drives the gene of interest is required. A fly line carrying the UAS-cDNA construct can then be crossed into any number of the existing fly lines that express GAL4 in a tissue-specific pattern. The progeny from such a cross can then express the gene of interest in the tissue of choice. These advances have allowed for the development of ectopic activation screens^{39,40} in which the UAS element is inserted randomly into the genome of the fly. Screening for interesting overexpression phenotypes can then be carried out. Such a screen might be used to find fly oncogenes by identifying genes whose overexpression can lead to the formation of tumors. Similarly, the UAS/GAL4 system can be used to express mammalian oncogenes or tumor suppressors ectopically during *Drosophila* development to assess their biological functions.

Another way to drive the heritable overexpression of a gene in a subset of tissues is the FLP-out technique⁴¹, which is based upon the introduction of the FLP/FRT system of yeast into the *Drosophila* genome⁴². FLP recombinase catalyzes the site-specific recombination between FLP recombination target (FRT) sites. An FLP-out construct consists of a constitutive promoter, followed by an FRT site, a marker gene with a poly-A (transcriptional terminator) site, a second FRT site and the cDNA of a gene of interest (Fig. 1). Once this construct is introduced into the fly genome, the expression of FLP recombinase by a heat-shock-inducible promoter will excise the DNA between the FRT sites, leading to a random assortment of cells that heritably overexpress the gene of interest. The combination of the FLP-out system with the UAS/GAL4 system can make this technique even more useful. If the FLP-out construct drives *Gal4* expression, this can, in turn, cause the heritable expression of any number of UAS-cDNA constructs. Neufeld *et al.* have used this approach to determine the effects of cell-cycle regulators, such as *Rbf* and *E2F*, on cell size, the cell cycle and cell division rate⁴³ (Fig. 1).

When an interesting phenotype is established by manipulations that either increase or decrease the activity of a gene of interest, the fly, like other model genetic organisms, can be used to isolate interacting genes by searching for second site mutations that suppress or enhance the original phenotype. Such so-called modifier screens are powerful tools to discover pathway components. For example, Delta was implicated to interact with the Notch receptor when mutations in Delta were recovered in a screen that could suppress the lethality associated with

FIGURE 1. The FLP/FRT system



(a) The expression of *GAL4* by the *Actin* promoter is disrupted by the presence of a transcription terminator (or a marker gene with a poly-A site). (b) Induction of the FLP recombinase gene causes site-directed recombination between the tandem FLP recombination target (FRT) sites in some cells, leading to the removal of the transcription terminator. (c) *GAL4* is now heritably and constitutively expressed in those cells that have removed the transcription terminator. *GAL4*, in turn, induces the expression of several transgenes whose expression is under the control of the *GAL4*-inducible promoter, UAS. For example, in the wing imaginal disc, UAS-green fluorescent protein (GFP) can be used to mark cells fluorescently that also express cancer-related genes, such as *E2F* (Ref. 43). Fluorescence-activated cell sorter (FACS) analysis can then be used to monitor changes in cell size and alterations in the cell cycle (DNA content). Furthermore, a comparison of the number of GFP-expressing cells in clones that do or do not express the gene of interest can be used to determine a change in cell division rate *in vivo*.

certain Notch alleles⁴⁴. Similarly, a screen for mutations that can modify the eye phenotype of *sevenless* mutations identified components of the *ras* pathway⁴⁵. Furthermore, if overexpression of a mammalian gene in the fly produces a phenotype that is suitable for a modifier screen, the fly system can be used to identify other pathway components without having to clone the fly version of the gene. Examining the effects of ectopically expressing human genes in the fly is becoming commonplace^{46,47}, and pathway components that are identified as a consequence of such analysis should be good candidates for mammalian tumorigenesis studies.

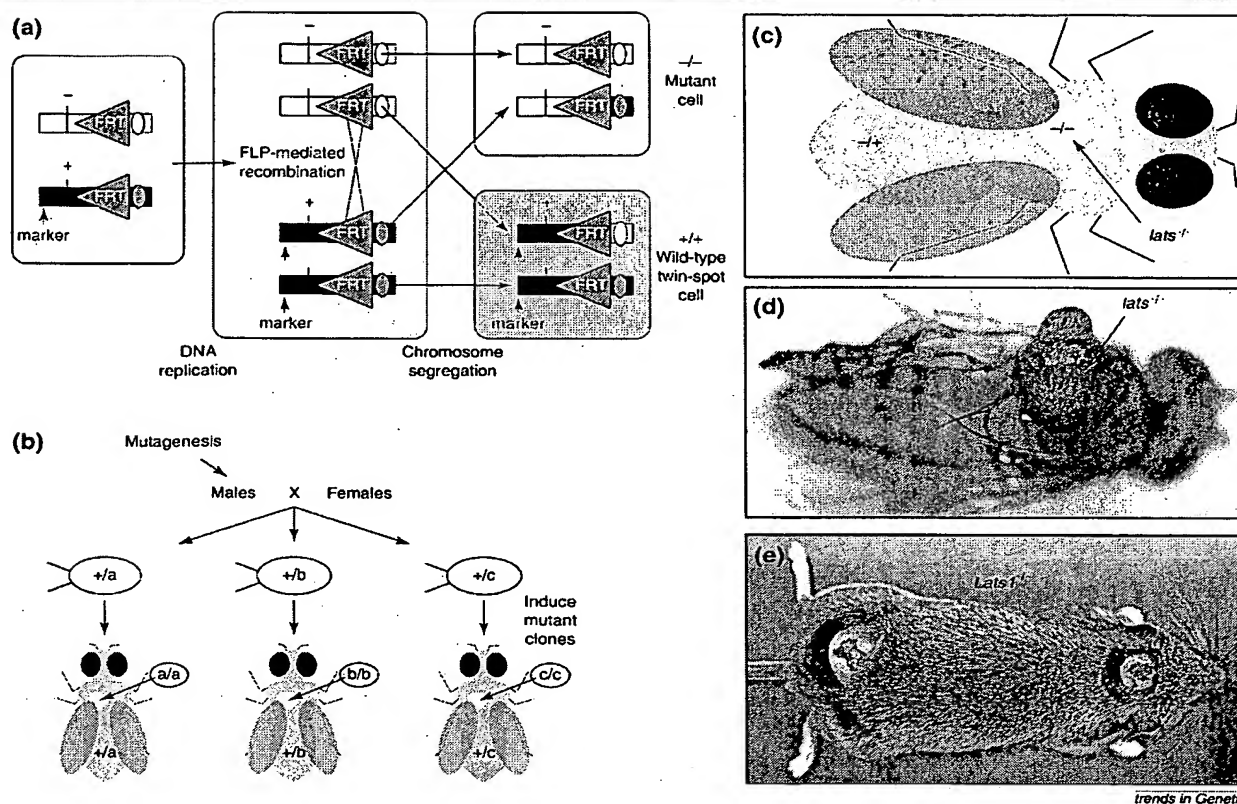
As a model genetic organism, like yeast or *C. elegans*, the entire genome of the fly can be screened systematically to identify any gene that, when mutated, affects the molecular mechanism of tumorigenesis. Such a large-scale mutagenesis approach is not practical in the mammalian model system. Furthermore, recent advances in genetic techniques that are unique to the fly system allow the fly to be used to more closely mimic cancer development in humans. Cancer in humans is a clonal phenomenon in which a somatic cell in an otherwise healthy patient loses growth control by mutation of one or more cancer-related genes. As many cancer-related genes are involved in essential processes during development and conventional screens only examine homozygous mutant animals, mutations of these genes will be missed when an animal dies before the cancer-related phenotype can be detected. A new type of genetic screen, termed a mosaic screen, more closely models the situation of a cancer patient and allows the isolation of novel tumor suppressor genes

(Fig. 2). In a mosaic screen, mutagenized males are crossed with normal females to produce a population of heterozygous embryos that each carry a distinct newly induced mutation (Fig. 2b). An FRT site has been inserted near the centromere on every major chromosome arm so that high frequency mitotic recombination can be induced between homologous chromosome arms in the developing heterozygous mutant animals to generate homozygous mutant clones in an otherwise healthy animal⁴⁸ (Fig. 2a, b). The high frequency of animals that carry mutant clones, in combination with autonomous cell markers, also allows for the direct examination of potential phenotypes in developing and internal tissues⁴⁸. Furthermore, because a mitotic recombination event also produces a wild-type twin-spot cell (or clone), the mutations that cause subtle phenotypes, such as a change in growth rate, can be identified by comparing the sizes of the twin-spot clones⁴⁹ (Fig. 1). The mosaic screen is also far more efficient than a standard genetic screen because it involves one generation instead of three^{48,50}. This technique has allowed the isolation of many novel genes that have been missed in conventional genetic screens^{49,51-53} (Fig. 2d).

The fly as a model of tumorigenesis

A mosaic screen for over-proliferation mutants has been used successfully to identify several novel tumor suppressors in flies, including the *large tumor suppressor* (*lats*; also known as *wts*) gene⁴⁹. Somatic cells mutant for *lats* undergo extensive proliferation and form large tumor outgrowths with morphological characteristics similar to

FIGURE 2. Mosaic screens to identify novel tumor suppressors



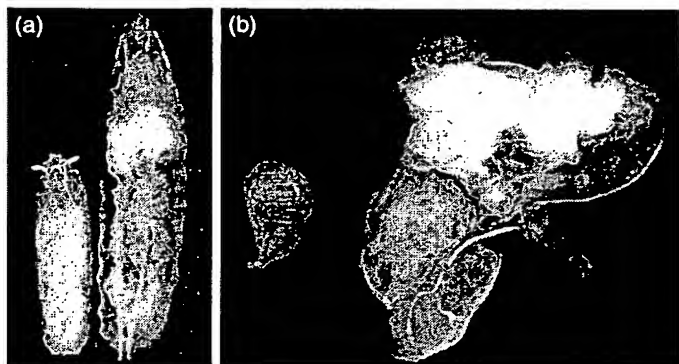
(a) Induction of homozygous mutant clones in heterozygous animals by FLP/FRT-mediated mitotic recombination. Homologous chromosomes are illustrated as white and black bars. High frequency mitotic recombination between chromosome arms can be induced at the FLP recombination target (FRT) sites (arrowhead) by the induction of FLP enzyme. After chromosome segregation, a daughter cell homozygous for the mutant gene (-/-) can be produced. The mutant cell also lacks the marker gene, allowing it to be distinguished from the wild-type twin-spot cell (+/+) or the heterozygous cells. (b) The FLP/FRT-mediated mitotic recombination can be used to generate mosaic animals that carry clones of cells that are homozygous for independently induced mutations. Mutations in tumor suppressors or other genes of interest can be identified in mosaic animals of the first generation. Mosaic flies are a good model for patients with cancer predisposition syndromes such as those heterozygous for mutated tumor suppressor genes. Loss of the wild-type copy of the tumor suppressor in these patients, or in the fly model (c), can lead to the development of a tumor (white patch). (d) The tumor suppressor function of *large tumor suppressor* (*lats*) is conserved in mammals as (e) mice mutant for the *Lats1* homolog develop soft-tissue sarcomas (see text).

those of human tumors, confirming that *Drosophila* can grow tumors that are comparable with those found in humans^{49,54} (Fig. 2d). Furthermore, mice that are deficient in the mouse *lats* homolog (*Lats1*) develop soft-tissue sarcomas and ovarian stromal cell tumors and exhibit heightened sensitivity to carcinogenic treatments⁵⁵ (Fig. 2e). This suggests that the underlying mechanism of tumorigenesis might be conserved as well, and a combination of fly and mammalian research has pointed to a conserved mechanism of *lats* function. The human homolog of the *lats* gene (*LATS1*) can be used to suppress tumor growth and rescue developmental defects in *lats* mutant flies⁴⁷. Further fly research and biochemical studies of *LATS1* indicate that *LATS* complexes with *CDC2* and negatively regulates the activity of the *CDC2/Cyclin A* complex^{47,55}. This result establishes a link between *lats* and a common target of the cancer process, the cell cycle. The demonstration that a previously uncharacterized tumor suppressor gene that was discovered in the fly can also act as a tumor suppressor gene in the mouse reinforces the value of using the fly

system to identify novel tumor suppressors. More importantly, the functional conservation of the *lats* gene suggests that many components of the *LATS* pathway are probably conserved between flies and mammals and the study of such genes in *Drosophila* will provide information that is directly relevant to tumorigenesis in humans.

Expanding role of the fly in cancer research

The future of the fly as a cancer research organism appears promising on several fronts. As the *Drosophila* and human genome projects advance, increasing numbers of cancer-related candidate genes will be identified. The fly will provide an excellent system for rapidly learning more about these genes. Studies in flies, together with similar work in other model genetic organisms, will provide information regarding the conserved molecular and biochemical properties of the cancer-causing molecules. For example, characterization of *gigas*, a fly homolog of a human tumor suppressor that is involved in the tuberous sclerosis complex (TSC), has shown that its mutation leads to the

FIGURE 3. *Lats* affects tissue size control

trends in Genetics

The loss of the *large tumor suppressor (lats)* gene in *Drosophila* leads to a disruption in tissue size control, resulting in giant larvae (a), (left: wild-type third instar larvae; right: *lats* mutant larvae) and dramatically enlarged imaginal discs (b), (left: wild-type third instar wing disc; right: *lats* wing disc).

development of giant polyploid cells, a phenotype that resembles human tuberous sclerosis tumors⁵¹. In addition, characterization of the fly homolog of phosphatase and tensin homolog (PTEN, also known as MMAC1) confirmed the results from mammals and *C. elegans* that PTEN functions in the insulin pathway and also revealed a role for PTEN in the regulation of cell size⁵⁶. On the other hand, even for those fly homologs of human oncogenes or tumor suppressors that have been studied, there is still much work to be done and much to learn. For example, *src* was one of the earliest mammalian oncogenes identified, yet the mechanisms of action of *src* in tumorigenesis remains largely unknown. The identification of the *Drosophila src* genes was the first attempt to study homologs of mammalian oncogenes in *Drosophila* and future studies of *Drosophila src* are likely to contribute significantly towards understanding the *src* pathway and the biological processes that it regulates⁵⁷⁻⁶⁰.

In areas in which the fly is not a traditional model system, such as cell-cycle checkpoint controls, researchers are discovering advantages to the fly system and are beginning to apply it to these issues with a fresh perspective⁶¹⁻⁶³. For example, a genetic screen for regulators of the radiation-damage checkpoint in imaginal discs has already recovered a novel gene that had not been identified in yeast screens (G. Rubin, pers. commun.).

Other processes that are important to cancer biology, but that have been less amenable to traditional approaches in molecular oncology, might be good candidates for future study in the fly. Although many tumor suppressor mutations have been tracked down through studies of familial cancer predisposition syndromes⁶⁴, mutations that are involved in metastasis have been harder to identify because they are late events. Experiments with tumors that are derived from *lethal giant larvae (lgl)* mutants have demonstrated that metastasis occurs in the fly^{65,66}. Flies that are homozygous for *lgl* mutations do not survive beyond the larval stage, but brain tumors that are transplanted from these mutants into normal adult flies exhibit metastasis by invading and spreading into distant

organs⁶⁶. Although *lgl* homologs have not yet been shown to be involved in human tumor metastasis, there are other lines of evidence that indicate that some of the biochemical mechanisms of metastasis, such as an increase in type IV collagenase, are conserved between flies and humans⁶⁵⁻⁶⁷.

Other genes that are involved in human tumor metastasis have been shown to have fly homologs. In the mammalian system, the *nm23* gene was discovered on the basis of its reduced expression in highly metastatic cell lines⁶⁸. This trend was confirmed in several types of human carcinomas and melanomas, and the suppressive effect of *nm23* on metastasis was demonstrated by overexpression in melanoma and breast carcinoma cells *in vivo*⁶⁹. Cloning of *nm23* identified it as a homolog of the *Drosophila* gene *abnormal wing discs (awd)*⁷⁰. Mutations in *awd* can cause abnormal tissue morphology and widespread aberrant differentiation that is analogous to the changes that occur in human malignant progression.

Future directions

The study of tumor suppressors in flies will probably lead to insights into some fundamental biological processes that are critical for the understanding of human cancer biology. For example, multicellular organisms require size-control mechanisms to determine when organ growth should be halted. Young imaginal discs transplanted into adult hosts stop growth once their normal size has been reached, suggesting that organ size control is both autonomous and genetic in origin⁷¹. Transplantation experiments in mice indicate that similar size-control mechanisms operate in mammals⁷². Furthermore, imaginal discs can undergo regeneration to form a normal-sized disc when a small region of the disc is surgically removed, suggesting that proliferating cells in a developing organ communicate with one another to maintain a constant organ size^{73,74}. It has also been shown that DNA replication and mitosis in growing imaginal discs occur in small, non-clonal cell clusters throughout the disc^{75,76}, which is consistent with the notion that proliferation is regulated by local cell-cell interactions. Mutations in the *lats* tumor suppressor have been shown to disrupt the cell-cell communication mechanism that controls the size of fly imaginal discs and thus allows the discs to grow unchecked (Fig. 3). Further study using the fly system might be useful in learning more about the molecular origins of the size-control mechanism and the context of its participation in cancer biology.

Because the fly does not have blood vessels, it cannot serve as a directly relevant model for studying the aspects of angiogenesis during tumor development. However, sections of fly tumors caused by mutations in the *lats* gene revealed that these tumors do contain lumen-like structures that might serve as channels for supplying nutrients for these fast-growing tissues^{45,77} (see Fig. 1). Interestingly, human tumors such as melanomas have now been shown to develop vascular channels that facilitate tumor perfusion independently of tumor angiogenesis⁷⁸. Although it is not clear whether these channel structures in insect and mammalian tumors are related, fly tumors might provide a powerful model to study the mechanisms and the biology of development of vascular structures in tumors.

The ongoing efforts of the fly community in developing and improving sophisticated genetic techniques are likely to further empower *Drosophila* as a model for cancer research.

For example, the mosaic screens performed so far have screened one chromosome arm at a time for mutations and tumorigenic events that require multiple hits on different chromosomes would have been missed. Future mosaic screens involving multiple chromosomes might identify new classes of tumor suppressors or mutations that contribute to metastasis. With its versatility in addressing many types of questions related to cancer biology and the demonstration of

its direct relevance to mammalian tumorigenesis, the fly system has a vital role to play in the future of cancer research.

Acknowledgements

We thank Karen Wehner and members of the Xu Lab for helpful comments. This work was supported by NIH grant RO1CA69408.

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